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Research Notes : United States : A greenhouse method of screening soybeans for resistance to Fusarium wilt

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1) A greenhouse method of screening soybeans for resistance to Fusarium wilt.

Fusarium wilt of soybean (causal organism: *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans.) has become an increasingly severe disease in the breeding plots at Gainesville and may be an undiagnosed or misdiagnosed problem in soybean production fields. At Gainesville, severity of Fusarium wilt, or a complex which includes *F. oxysporum*, has reduced yields in some plots to nearly zero. However, resistance to the disease has been observed in the breeding material, particularly in the "vegetable type" families which have 'Late Giant' as a parent, and in other families to a lesser extent. Variability in symptom expression within and among families is confounded with differences in the inoculum levels encountered throughout the field. Diseased and dead plants occur in patches of irregular shape. This field variability hinders progress in the selection of resistant types. Additionally, interactions between *F. oxysporum* and other soybean pests, particularly nematodes, may further obscure accurate selection. A greenhouse screening method would greatly facilitate the selection of resistant types by reducing the effects of variability found in field nurseries. This report describes some results of our efforts to screen soybeans for resistance to Fusarium wilt in the greenhouse.

Materials and Methods: Samples of discolored vascular tissue from field-grown soybeans were surface sterilized with 0.5% NaOCl and plated on acid PDA (aPDA) and peptone PCNB agar. After 8 days, clean cultures were transferred to carnation leaf water agar and aPDA to induce sporulation and for identification. Positive colonies of *F. oxysporum* were mass transferred to aPDA and kept at 21°C in constant light for inoculum production. After 2 weeks, the plates were rinsed with sterile distilled water, the propagules collected, and the suspension adjusted to 6×10^4 propagules/ml with a hemacytometer. The propagule suspension was added to sterilized greenhouse mix and preliminary pathogenicity testing began. Reisolation from diseased plants confirmed pathogenicity of the isolate. Soil from the preliminary tests was sampled and assayed to determine the final concentration of *F. oxysporum* propagules by soil dilution plating on a selective medium described by Komada (1975).

Five soybean lines were used in this experiment: Late Giant, F80-6717, Co82-645, D78-4668, and 'Yelredo'. Late Giant is a black-seeded "vegetable

type" of uncertain origin that has shown resistance to Fusarium wilt in the field and the preliminary greenhouse tests. F80-6717 is a black-seeded breeding line that has Late Giant in its parentage. Co82-645 is a breeding line from CR SEEDS with resistance to soybean cyst (*Heterodera glycines*) race 3 (SCN) and root knot (*Meloidogyne incognita* and *M. arenaria*) (RKN) nematodes. D78-4668 is a breeding line supplied by E. E. Hartwig, Stoneville, MS, from which *F. oxysporum* has previously been isolated. Armstrong and Armstrong (1950) have reported Yelredo to be very susceptible to Fusarium wilt.

Test design was a Latin Square with one square for each of three inoculum levels. Undiluted, infested soil from the preliminary tests was used as the base level of inoculum (13500 propagules/g soil). Samples of this soil were diluted with unsterilized greenhouse mix to levels of 10% and 5% of the base level. Six seeds of each soybean line were planted 2.5 cm deep in 15 cm plastic pots in the greenhouse and thinned at 7 days to three uniform plants/pot. One pot of each line was planted in unsterilized greenhouse mix as controls. Temperature and humidity were not controlled, but were within the range that permitted rapid plant development. Visual ratings were made weekly beginning 14 days after planting and ending 35 days after planting. Ratings were on a 1 to 5 scale with 1 equal to the noninoculated control, intermediate scores reflecting increasing chlorosis, and 5 indicating severe necrosis, particularly of the expanding trifoliolates.

Results and Discussion: Mean scores for 14 and 35 days from planting were not significantly different for any of the three inoculum levels. Mean scores for reaction of the five lines to the three levels of *F. oxysporum* at 21 and 28 days from planting are presented in Table 1.

The best separation of mean scores for all lines was obtained 21 days after planting in soil containing 1350 propagules/g soil. This was also the combination with the largest difference between the highest and lowest mean scores. Late Giant consistently had the lowest, or equal to the lowest, mean scores. D78-4668 was scored significantly more susceptible than Late Giant only at 21 days from planting and 1350 propagules/g soil. F80-6717 received significantly more susceptible scores than Late Giant only at the 1350 propagules/g soil inoculum level at both 21 and 28 days from planting, and only at 21 days from planting could F80-6717 be distinguished from the susceptible check, Yelredo. Thus, F80-6717 probably did not receive the full gene complement for resistance from Late Giant.

Table 1. Mean score of soybean lines grown in greenhouse soil infested with *Fusarium oxysporum*

Line	21 days from planting — Propagules/g soil — 13500	1350	675	28 days from planting — Propagules/g soil — 13500	1350	675
	Score[+]					
Late Giant	2.6 a	2.0 a	1.0 a	3.6 a	3.2 a	1.8 a
D78-4668	3.2 ab	2.6 b	1.0 a	4.0 ab	3.2 a	2.0 a
F80-6717	3.2 ab	3.0 bc	1.0 a	4.2 ab	4.0 b	2.4 a
Co82-645	3.4 b	3.4 cd	1.6 b	4.4 b	4.0 b	2.4 a
Yelredo	3.8 b	3.6 d	1.2 ab	4.6 b	4.0 b	2.2 a

[+]From 1 = noninoculated control to 5 = severe. Means in columns followed by the same letter not significantly different at 5% level based on Duncan's New Multiple Range Test.

Co82-645 was scored significantly more susceptible than Late Giant in all but one combination of reading date and inoculum level (28 days from planting and 675 propagules/g soil). Ross (1965) has sown a detrimental interaction of *F. oxysporum* and SCN or RKN versus a weakly pathogenic reaction of *F. oxysproum* on nematode-susceptible soybeans in the absence of nematodes. In a soil assay performed after completion of this study, no nematodes were found in the infested soil. The severe damage from *F. oxysporum* occurred independently of nematodes on both nematode-susceptible and -resistant soybeans.

The susceptibility of Yelredo to Fusarium wilt was confirmed. On both reading dates, at 13500 and 1350 propagules/g soil, Yelredo had the highest, or equal to the highest, mean scores of all lines tested.

Using this greenhouse method, we were able to reliably separate resistant and susceptible soybean lines. Additionally, the short screening duration provides rapid determinations of reaction. With modifications, this method could prove useful in inheritance and other genetic studies. Further testing is in progress to identify better adapted sources of resistance, develop a nonsubjective scoring technique, and modify the method to allow more detailed evaluations.

References

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